

Try to saturate 3' end with
 Tag (see P29)

Project N _____
 Book N _____

ag N _____

Tag assay mix (P557)
 except no activated DNA

[A]

(40 Rxns)

0.5 M Tris pH 8.3

100

✓

1 M MgCl₂

4

✓

3 M KCl

33.3 μ l

✓

1 ATP-G-TP 10 μ l each

40

✓

2 ³²P dCTP

5.1 4.2 μ l

✓

H₂O

1.219 ml

✓

① 1.4 ml 385

②

(use 3.5 μ l / 5 μ l rxn)

(same as P17)

3.0 μ l 500 μ mol nt/ μ l

13.3 μ l

66.7 μ l

11 Rxns

(4.5 μ l / 5 μ l)

0.165 μ g (μ l)

① = 0.2 μ g DNA / 50 μ l

② = 1 μ g DNA / 50 μ l

(= 5 μ mol nt / 5 μ l)

H₂O

96.7

41.5

49.5

49.5

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

4.5 μ l →

4.5 μ l →

total units

EKBTI

28 5

5

0.4

6 5

5

0.8

12.5 5

5

1.5

25 5

5

3.125

50 5

5

6.25

100 5

5

12.5

200 5

5

25

400 5

5

50

800 5

5

100

1600 5

5

200

3200 5

5

6400 5

5

12800 5

5

25600 5

5

51200 5

5

102400 5

5

204800 5

5

409600 5

5

819200 5

5

1638400 5

5

3276800 5

5

essed & Understood by me,
 Erika Polak

Date
 10/24/94

Invented by
 Recorded by

Dat
 10-18-94

T Pag No. _____

2 min at 74°C → add 10 μ l EDTA
 spot 40 μ l on GFC

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units / 50 μ l		pmol	
0.4	1	133.00	2.6
0.8	2	248.00	4.9
1.6	3	264.00	5.2
3	4	470.00	9.2
6	5	633.00	12.5
12	6	886.00	17
25	7	991.00	19.5
50	8	995.00	19.6
100	9	999.00	19.7
200	10	883.00	17.4
0.4	11	2146.00	42
0.8	12	3847.00	73
1.6	13	6695.00	132
3	14	12077.00	238
6	15	17179.00	339
12	16	17333.00	342
25	17	22279.00	440
50	18	22941.00	452
100	19	23863.00	471
200	20	24510.00	477
500	21	92.00	
1000	22	304197.00	76 CPM/pmol

need time course at high and low [Tag]
to see if lag plays a role. In a PCR with
15-30 min elongation time, effect of lag would
be minimized

Results per Tag spot = $100,000$ u/mg
25 units = 50 nm Tag
50 μ l

Both plots (0.2 and 1.0 μ g DNA) saturate ~ 50 nm Tag
suggesting an equilibrium effect of pol DNA binding
rather than titration of pol at 1 pmol/mg ~~pol~~
1 μ g DNA, saturation at 10 u / 0.42 pmol circles

for 200,000 u/mg tag

1 unit Tag = 0.053 pmol molecules \sim 1.1 Tag/circle

1 μ g mp19 \Rightarrow $\frac{1 \times 10^{-6} \text{ g}}{(330 \text{ g mole wt})(7250 \text{ bp})} = 0.42 \text{ pmol circle}$

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Witnessed & Understood by me,

D. S. S. S. S.

Date

10/24/94

Invented by

Rec rded by

Dat

10-77-94